

I. AMENDMENTS TO THE SPECIFICATION

Please replace paragraphs as filed with the following replacement paragraphs:

[00154] Examples of cleavable oligonucleotides which contain two reverse U (rU) linkers and have been synthesized on a chip are as follows:

Probe	Pu1	PS1	PU2	PS2
IL6_T7	5'CAAGGATCTTACCGCTGTTGtgaggagacttgccctggtgrUTAATACGACTCACTAT AGGtctgcaggaactggatcaggrU (SEQ ID NO:1)			
CYP11A_t7	5'CAAGGATCTTACCGCTGTTGgtgaccctgcagagatatctrUTAATACGACTCACTA TAGGgttccggaagtaggtgatgrU (SEQ ID NO:2)			
ATP2A1_T7	5'CAAGGATCTTACCGCTGTTGgattggcattgccatgggatrUTAATACGACTCACTAT AGGtccacagcagctacgatggrU (SEQ ID NO:3)			
IL6_Nick	5'caaggatctt accgctgttg tgaggagact tgcctggtgn cgctccagac ttgagtccga tctgcaggaa ctggatcaggrU (SEQ ID NO:4)			
CYP11A_Nick	5'CAAGGATCTTACCGCTGTTGgtgaccctgcagagatatctrUCGCTCCAGACTTGAG TCCGAgttccggaa gtaggtgatgrU (SEQ ID NO:5)			
ATP2A1_Nick	5'CAAGGATCTTACCGCTGTTGgattggcattgccatgggatrUCGCTCCAGACTTGAG TCCGAtccacagcagctacgatggrU (SEQ ID NO:6)			

[00164] Different strategies can be used to release or cleave oligonucleotides synthesized on a solid substrate from that substrate. The cleavage efficiency of three different linkers was examined to determine the preferred linker(s) for cleaving oligonucleotides from a solid substrate (rU is 5'-phosphoramidite with 2'-acetyl and 3'DMT; U is 3'-phosphoramidite with 2'fpm and 5'-DMT; and dU is 2'-deoxyuridine). To begin, the following oligonucleotides were synthesized using an Expetide™ DNA synthesizer and standard phosphoramidite chemistry:

Sequence A 3' ttttttttttrugtccacagcatccga-fam-5' (SEQ ID. NO:7)

Sequence B 3' ttttttttttugttccacagcatccga-fam-5' (SEQ ID. NO:8)

Sequence C 3' ttttttttttdugtccacagcatccga-fam-5' (SEQ ID. NO:9)

[00168] The GFP gene is 714 base pairs (bp) long. Suitable subchains (computational fragmentation) for the assembly of the GFP gene were selected, and oligonucleotides between 40 and 47 nucleotides long were synthesized on a chip using the methods outlined above. The complete set of 34 GFP subchains synthesized on a chip are as follows:

GFP-F2	ATGAGTAAAG GAGAAGAACT TTCTACTGGA GTTGTCCTCA TTCTTG	SEQ ID NO:10
GFP-F3	TTGAATTAGA TGGTGATGTT AATGGGCACA AATTTTCTGT CAGT	SEQ ID NO:11
GFP-F4	GGAGAGGGTG AAGGTGATGC AACATACGGA AAACCTACCC T	SEQ ID NO:12
GFP-F5	TAAATTTATT TGCACACTG GAAAACTACC TGTTCATGG CCAA	SEQ ID NO:13
GFP-F6	CACCTGTAC TACTTTCTCT TATGGTGTT AATGCTTTTC AAGATA	SEQ ID NO:14
GFP-F7	CCCAGATCAT ATGAAACGGC ATGACTTTTT CAAGAGTGCC AT	SEQ ID NO:15
GFP-F8	GCCCGAAGGT TATGTACAGG AAAGAATAT ATTTTCAAA GATG	SEQ ID NO:16
GFP-F9	ACGGGAAC TAAGACACGT GCTGAAGTCA AGTTTGAAGG T	SEQ ID NO:17
GFP-F10	GATACCCTTG TTAATAGAAT CGAGTTAAAA GGTATTGATT TTAAG	SEQ ID NO:18
GFP-F11	AAGATGGAAA CATTCTTGGA CACAAATTGG AATACAACTA TAACTC	SEQ ID NO:19
GFP-F12	ACACAATGTA TACATCATGG CAGACAAACA AAAGAATGGA ATCAA	SEQ ID NO:20
GFP-F13	AGTTAACTTC AAAATTAGAC ACAACATTGA AGATGGAAGC GTTCA	SEQ ID NO:21
GFP-F14	ACTAGCAGAC CATTATCAAC AAAATACTCC AATTGGCGAT GG	SEQ ID NO:22
GFP-F15	CCCTGTCCTT TTACCAGACA ACCATTACCT GTCCACACAA T	SEQ ID NO:23
GFP-F16	CTGCCCTTTC GAAAGATCCC AACGAAAAGA GAGACCACAT G	SEQ ID NO:24
GFP-F17	GTCCTTCTTG AGTTTGTAAC AGCTGCTGGG ATTACACATG GC	SEQ ID NO:25
GFP-F18	ATGGATGAAC TATACAAATA GCATTCTAG AATTGACTCT ATAGTG	SEQ ID NO:26
GFP-R1	TGAAAAGTTC TTCTCCTTTA CTCAT	SEQ ID NO:27
GFP-R2	ATTAACATCA CCATCTAATT CAACAAGAAT TGGGACAACT CCAG	SEQ ID NO:28
GFP-R3	CATCACCTTC ACCCTCTCCA CTGACAGAAA ATTTGTGCC	SEQ ID NO:29
GFP-R4	TTCCAGTAG TGCAAATAAA TTAAAGGGTA AGTTTCCGT ATGTTG	SEQ ID NO:30
GFP-R5	ATAAGAGAAA GTAGTGACAA GTGTTGGCCA TGGAACAGGT AGT	SEQ ID NO:31
GFP-R6	GCCGTTTCAT ATGATCTGGG TATCTTGAAA AGCATTGAAC ACC	SEQ ID NO:32
GFP-R7	CCTGTACATA ACCTTCGGGC ATGGCACTCT TGAAAAAGTC AT	SEQ ID NO:33
GFP-R8	ACGTGTCTTG TAGTTCCCGT CATCTTTGAA AAATATAGTT CTTT	SEQ ID NO:34
GFP-R9	CGATTCTATT AACAAGGGTA TCACCTTCAA ACTTGACTTC AGC	SEQ ID NO:35
GFP-R10	TGTCCAAGAA TGTTTCCATC TTCTTTAAAA TCAATACCTT TAACT	SEQ ID NO:36
GFP-R11	TGCCATGATG TATACATTGT GTGAGTTATA GTTGATTCC AATTG	SEQ ID NO:37
GFP-R12	TTGTGTCTAA TTTTGAAGTT AACTTTGATT CCATTCTTTT GTTTGTC	SEQ ID NO:38
GFP-R13	TTGTTGATAA TGGTCTGCTA GTTGAACGCT TCCATCTTCA ATG	SEQ ID NO:39
GFP-R14	TGTCTGGTAA AAGGACAGGG CCATCGCCAA TTGGAGTATT	SEQ ID NO:40
GFP-R15	GGGATCTTTC GAAAGGGCAG ATTGTGTGGA CAGGTAATGG T	SEQ ID NO:41
GFP-R16	CTGTTACAAA CTCAAGAAGG ACCATGTGGT CTCTCTTTTC GTT	SEQ ID NO:42
GFP-R17	TGCTATTTGT ATAGTTCATC CATGCCATGT GTAATCCCAG CAG	SEQ ID NO:43

[00169] Additionally, the following two control oligonucleotides (Puc2PM- perfect match and Puc2MM- mismatch) were also synthesized on the chip using the methods outlined above:

PUC2PM	CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA	<u>SEQ ID NO:44</u>
PUC2MM	CTGGCAGTAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA	<u>SEQ ID NO:45</u>

[00179] One method for releasing or cleaving synthesized oligonucleotides from a solid substrate is an enzymatic approach involving the use of restriction endonuclease (R.E.) enzymes to selectively and specifically cleave desired oligonucleotides from the substrate surface. To test this approach, the Dpn II R.E. enzyme was used to cleave two complementary oligonucleotide DNAs, the first oligo being GFP-F2Part 5'-CACTGGAGTTGTCCCAATTCTTGgacgccc-3' (SEQ ID NO:46) and the second one being DpnIISite 5'-ggccgacCAA-3' (SEQ ID NO:47). Since II enzyme recognizes and cleaves the sequence 5'-[^]GATC-3', the isolation of clean oligonucleotides was expected after digestion with the enzyme. Our initial test on the digested oligonucleotides in solution phase was successful. In the experiment, two oligonucleotides were mixed at a molar ration of 1:5 (GFP-F2Part:DpnIISite) and incubated with or without Dpn II enzyme at 37°C. These reactions were analyzed at various time points with CE (capillary electrophoresis, 10% polyacrylamid gel with 7 M urea). As shown in Figure 23, approximately 80% of the longer oligonucleotides were cut by Dpn II in 1 hour. This experiment demonstrates the efficient release of synthesized oligonucleotides from the substrate surface through the use of R.E. enzymes.

[00180] In other embodiments of the present disclosure, an oligonucleotide sequence can be synthesized such that it will anneal itself, thereby forming a duplex oligonucleotide with a hairpin loop. The duplex DNA can then be digested with an enzyme, for example a R.E. enzyme, to form double-stranded DNA that can be ligated to other double-stranded DNA and/or oligonucleotides. To demonstrate the ability of a R.E. enzyme to digest a synthesized oligonucleotide that anneals to itself, the following oligonucleotide sequences with FAM label (DEFINE FAM) were synthesized on a chip with a regular DMT chip surface:

ePM-40	FAM- CTGGCAGCAGCCACTTTAACTATGCGGCATTAACTATGCGATCGGCCTTTTGGCCGATCGCAT AGTTAAATGCCGCATAGTTAAAGTGGCTGCTGCCAG	<u>SEQ ID NO:48</u>
ePM-20	FAM- CTGGCAGCAGCCACTTTAACTATGCGGCATTAACTATGCGATCGGCCTTTTGGCCGATCGCAT AGTTAAATGCCGCATA	<u>SEQ ID NO:49</u>

eMM-40	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTACATGCCGCATAGTTAAAGTGGCTGCTGCCAG	<u>SEQ ID NO:50</u>
eMM-40-2	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTACATGCCGCATAGTTAAAGTGGCCGCTGCCAG	<u>SEQ ID NO:51</u>
eMM-20	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTACATGCCGCATA	<u>SEQ ID NO:52</u>
eD-40	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTAATGCCGCATAGTTAAAGTGGCTGCTGCCAG	<u>SEQ ID NO:53</u>
eD-40-2	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTAATGCCGCATAGTTAAAGTGGCGCTGCCAG	<u>SEQ ID NO:54</u>
eD-20	FAM-CTGGCAGCAGCCACTTTAACTATGCGGCATTTAACTATGCGATCGGCCTTTTGGCCGATCGCATAGTTAATGCCGCATA	<u>SEQ ID NO:55</u>

[00183] This efficiency of the PGA chemistry utilized in the present disclosure also results in the ability of this chemistry to generate synthetic oligonucleotide sequences that are significantly longer than those that could be synthesized using previously disclosed methods. A programmable light-directed synthesis system was used to synthesize oligomers up to 100 nucleotides in length on a microfluidic array chip. The oligonucleotides synthesized on a chip were as follows:

[illegible]

Pu2P M-15	CTGGCAGCAGCCACT	SEQ ID NO:73
Pu2M M-100	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGCGGCATTAACATGCGGCATTTAACTATGC	SEQ ID NO:74
Pu2M M-95	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGCGGCATTAACATGCGGCATTTAAC	SEQ ID NO:75
Pu2M M-90	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGCGGCATTAACATGCGGCAT	SEQ ID NO:76
Pu2M M-85	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGCGGCATTAACATGC	SEQ ID NO:77
Pu2M M-80	CTGGCAGTAGCCACTTTAACTATGCGGCAT TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTAAC	SEQ ID NO:78
Pu2M M-75	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGCGGCAT	SEQ ID NO:79
Pu2M M-70	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACT ATGC	SEQ ID NO:80
Pu2M M-65	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAAC	SEQ ID NO:81
Pu2M M-60	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCAT	SEQ ID NO:82
Pu2M M-55	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:83
Pu2M M-50	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAAC	SEQ ID NO:84
Pu2M M-45	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGCGGCAT	SEQ ID NO:85
Pu2M M-40	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:86
Pu2M M-35	CTGGCAGTAGCCACTTTAACTATGCGGCATTTAAC	SEQ ID NO:87
Pu2M M-30	CTGGCAGTAGCCACTTTAACTATGCGGCAT	SEQ ID NO:88
Pu2M M-25	CTGGCAGTAGCCACTTTAACTATGC	SEQ ID NO:89
Pu2M M-20	CTGGCAGTAGCCACTTTAAC	SEQ ID NO:90
Pu2M M-15	CTGGCAGTAGCCACT	SEQ ID NO:91
Puc2D -100	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCATTAACATGCGGCATTTAACTATGC	SEQ ID NO:92
Puc2D -95	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCATTAACATGCGGCATTTAAC	SEQ ID NO:93
Puc2D -90	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCATTAACATGCGGCAT	SEQ ID NO:94
Puc2D -85	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCATTAACATGC	SEQ ID NO:95
Puc2D -80	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCATTAAC	SEQ ID NO:96
Puc2D -75	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGCGGCAT	SEQ ID NO:97
Puc2D -70	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTA TGC	SEQ ID NO:98
Puc2D -65	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAAC	SEQ ID NO:99
Puc2D -60	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCAT	SEQ ID NO:100
Puc2D -55	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:101
Puc2D -50	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCATTTAAC	SEQ ID NO:102
Puc2D -45	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGCGGCAT	SEQ ID NO:103
Puc2D -40	CTGGCAGAGCCACTTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:104

Puc2D -35	CTGGCAGAGCCACTTTAACTATGCGGCATTTAAC	SEQ ID NO:105
Puc2D -30	CTGGCAGAGCCACTTTAACTATGCGGCAT	SEQ ID NO:106
Puc2D -25	CTGGCAGAGCCACTTTAACTATGC	SEQ ID NO:107
Puc2D -20	CTGGCAGAGCCACTTTAAAC	SEQ ID NO:108
Puc2D -15	CTGGCAGAGCCACT	SEQ ID NO:109
Stem- 85	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:110
Stem- 80	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:111
Stem- 75	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:112
Stem- 70	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:113
Stem- 65	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:114
Stem- 60	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:115
Stem- 55	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:116
Stem- 50	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:117
Stem- 45	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:118
Stem- 40	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:119
Stem- 35	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:120
Stem- 30	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:121
Stem- 25	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:122
Stem- 20	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:123
Stem- 15	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:124
Stem- 10	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:125
Stem-5	TTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGCGGCATTTAACTATGC	SEQ ID NO:126

[00184] The oligonucleotides were designed to contain a 15-mer probe (CTGGCAGCAGCCACT) (SEQ ID NO:73) at their 5'-end and connected to variable sizes of non-probe sequence from 0 to 85 nucleotides in length. Additionally, a single base mismatch 15-mer (CTGGCAGTAGCCACT) (SEQ ID NO:91) probe and a single base deletion 14-mer (CTGGCAGAGCCACT) (SEQ ID NO:109) probe were also synthesized on the chip as control sequences. Oligonucleotides from 5 to 100 nucleotides in length were synthesized on the chip, and the two control sequences were arranged side by side in the array for comparison purpose. After the oligomers were synthesized on the array chip, the chip was deprotected with EDA at room temperature for 2 hours and fill with 6xSSPE buffer. The 15 nucleotide target

oligonucleotide labeled with a Cy3 dye was hybridized to the chip in 6xSSPE for 2 hours at room temperature, and the chip was subsequently washed with 0.001xSSPE buffer. As illustrated in Figure 25 and shown in Figure 26, the presence of fluorescence on the chip after the hybridization assay demonstrates that 100-mer oligonucleotides were synthesized on the chip. Additionally, the fluorescence intensity profile indicated a stepwise yield of 98.5% for the synthesis of these long oligonucleotides, which is a significant improvement over known methods for synthesizing oligonucleotides on an array chip. In another experiment, a comparison of the per step yield for oligonucleotides 15 to 100 nucleotides in length on a dual chip demonstrated an even higher stepwise yield of 98.9% and 99.1% (figure 27).

[00187] The disclosed methods for generating pools of oligomers can also be used to generate an RNAi (RNA interference) chip. 252 oligonucleotides were generated on an RNAi chip using the methods previously outlined, with each oligonucleotide synthesized containing a SAP1 sequence (TGCAGTTAGCTCTTCASAT) (SEQ ID NO:128) at the 3' end, a variable RNAi specific sequence in the middle (22 nucleotides in length), and a T7 promotor sequence CCTATAGTGAGTCGTATTA) (SEQ ID NO:129) at the 5'-end (total length about 60 nucleotides). In order to cleave the oligonucleotides from the chip, reverse-U was incorporated into the 3'-end of all oligonucleotides. Additionally, the same two control oligonucleotides (puc2PM- (SEQ ID NO:44-perfect match) and Puc2MM- (SEQ ID NO:45-mismatch) as disclosed in example 3 were also synthesized on the RNAi chip. The quality of the oligonucleotides synthesized on the RNAi chip was also analyzed by hybridization with Cy3 labeled 15-mer Puc2 target as outlined in Example 3.

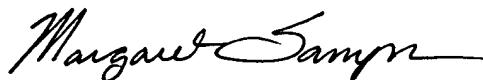
II. ADDITION OF SEQUENCE LISTING

Please place the attached paper copy of the "Sequence Listing" in the captioned application beginning as a new page after the "Abstract of the Disclosure."

III. CONCLUSION

Applicants believe that a full and complete reply has been made to the Request to Comply with Sequence Rules and, as such, the present application is in condition for allowance. Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,



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